Peer Review File

Manuscript Title: Escape of SARS-CoV-2 501Y.V2 from neutralization by convalescent plasma

Editorial Notes:

Reviewer Comments & Author Rebuttals

Reviewer Reports on the Initial Version:

Referee #1 (Remarks to the Author):

Cele et al report on the neutralizing activity of 6 individuals that were infected with B.1 during the first wave of infection in South Africa. Five of the individuals were female one was on supplemental oxygen all were collected 26-30 days after symptom onset.

The plasma from the B.1 infected individuals was assayed against 501Y.V2, that carries the 417/484/501 RBD mutations in a microneutralization live virus focus forming assay. The data is presented as a ratio between number of foci in the presence and absence of plasma. IC50s against first wave virus varied from 4,000-10,000. The combined data for two different 501Y.V2 variants varied from undetectable to 6-fold lower than B.1. Although the results are consistent with a series of reports showing that the SA variant is less sensitive to convalescent plasma, the small number of individuals examined, the bias of the cohort to females, and the lack of longitudinal data makes the data difficult to evaluate in the overall context of the pandemic. In conclusion the manuscript feels limited and the data set insufficient to make reliable conclusions.

Additional Problems:

1. The authors “combined the data for both 501Y.V2 variants” before calculating neutralization parameters. As neutralization results for those variants look very different (Fig. S2 B/D), it is not clear why this was done. The authors could have calculated independent ratios to neutralization of the matched 1st wave variant. This would have been especially important since one of the two variants contained a furin cleavage site mutation.
2. In addition, it is not clear how the data was combined. The curves in Fig. S2 show overall lower maximum values than those in Figure 2.
3. It is unclear whether the 1st wave neutralization profiles were also combined? If so, how did the authors get from Figure S2 A/C to Figure 2B? The yellow curve for example has its maximum at 0.7 in S2, but at 0.9 in Figure 2. The control curve has its maximum at 1.0/0.8 in Fig. S2, but at 1.2 in Figure 2.
4. There is no doubt that 501Y.V2 neutralization is strongly impaired when looking at the neutralization curves, but given the neutralization curves shown here, it is not clear how accurate the reported titer ratios are (1st wave vs. 501Y.V2 neutralization).
5. For the 1st wave virus, the plasma was not diluted out sufficiently to see maximum infection (0% neutralization). This can make the curve-fitting imprecise. For the yellow curve for example, the neutralization range covered is only around 40-85% and this range was then used to define NT50. This effect is even more pronounced when looking at the data in Fig. S2, showing data prior “combining”.
6. For 501Y.V2 none of the curves even reaches close to 50% neutralization and titers were consequentially extrapolated from curve fits. This extrapolation might make the titer quite inaccurate which will consequently strongly affect the ratios (neutralization 1st wave vs. neutralization 501Y.V2). The lowest dilution tested is 1:100, the lowest titer reported is 1:3. If this

titer in reality is e.g. 1:12, this will reduce the ratio of 1st wave to 501Y.V2 from 200- to 50-fold.

Minor points:
1. Neutralization activity of plasma should be expressed as neutralization titers (NTx) and not as inhibitory concentrations (ICx). The parameter measured is the plasma dilution at which 50 % neutralization was observed, therefore neutralization titers are usually expressed as reciprocal plasma dilutions, e.g. NT50=100 if 50% neutralization was observed at 1:100 plasma dilution.
2. Line 85: 1x10-3 equals 1:1000
3. Figure 2E: colors are swapped

Referee #2 (Remarks to the Author):

In their manuscript Cele and colleagues describe the neutralizing capacity of six serum samples against two isolates of B.1.351. Of course, the manuscript is highly significant and the information important, but there are many points that need the authors' attention.

Major points

1) The very limited number of samples and the absence of post-vaccination serum limit the significance of the findings considerably.

2) One isolate 'escapes' neutralizing antibody responses much better than the other one. That isolate has also a much bigger plaque phenotype. Better growth characteristics in vitro can easily skew results of neutralization assays but may have no impact in vivo. It would be very very important to make sure that the virus growth kinetics allow simple cross-comparisons. The best would be to use a control that should inhibit all three isolates at a similar level, e.g. remdesivir. That would give much more confidence in the findings. If remdesivir inhibits the HV001 also much less than HVdF002, then it becomes clear that this is caused by an assay artifact.

3) The panels in Figure S2 should be moved to Figure 2 to make sure that these differences are abbreviated.

4) Inhibition based on serum dilutions should be reported as ID50, not IC50. The specific antibody concentration in serum is not known (text and figure 2).

5) The way the inhibition curves are shown is confusing. The dilutions (1:100 etc.) should be shown on the x-axis. % inhibition should be shown on the y-axis.

6) The trial results from J&J and Novavax in RSA should be discussed.

Minor points

1) Line 48: Define 'ELISA'

2) Line 57, 229: 'spike', not 'Spike'

3) Line 85: Are we sure this calculation is correct? In my book 1x10-3 is 1:1000.

4) Figure 2E: With all the variation shown in B and C, how can the error bars here be so low?

5) Line 125: Please provide a reference for the increase of reinfections.

6) Line 126/127: After the second vaccination neutralizing antibody titers are consistently in the top 25% of what is seen with natural infection. Also, based on the titers shown, the convalescent

serum used in the study was not very potent.

7) Many abbreviations, especially in the methods part, are not defined.

8) Line 198: ‘biosafety’, not ‘Biosafety’

9) The way the assay is run the serum concentration used to incubate with the virus before infection of cells is not consistent with the serum concentration present in the overlay during the assay. This is unusual and should be pointed out.

Referee #3 (Remarks to the Author):

This is a very important contribution for our understanding of whether the newly arising variants of SARS-CoV-2 escape immunity to the original virus. The data suggest that the polyclonal plasma antibodies generated in an immune response to the original SARS-CoV-2 virus are significantly less efficient in blocking infection of target cells, when comparing the 501Y variant to the original virus. The finding adds a decisive facet to the recent reports, published as preprints (refs #12 -14 of ms), that monoclonals of convalescent COVID-19 patients, or serum from one such patient, are inefficient in neutralising E484 variants, which would include 501Y. Here, the authors go beyond that and demonstrate that polyclonal convalescent serum of several COVID-19 patients shows little to no efficacy to block infection of VERO E6 cells by the 501Y variant virus. Presumably via the spike protein binding its target ACE2, although this has not been shown directly. Although still epidemiological data are missing, demonstrating that indeed immune responses to the original SARS-CoV-2, or to the existing vaccines, do or do not protect from infection with the 501Y variant, the evidence presented in this manuscript is alarming and should initiate and justify the immediate development of adapted vaccines. In the current situation, the work is of utmost relevance and I highly recommend acceptance, pending a few minor revisions (see below).
Technically the approach is pretty straightforward. The authors describe outgrowth and isolation of two 501 variants carrying a number of mutations in the RBD and NTD domains of the spike protein, including E484K and N501Y. They then use plasma from blood of convalescent patients, collected at about 1 month after onset of symptoms, diluted at 1:100 to 1:1600, mix it with 50 "focus-forming units" of virus and infect Vero E6 cells for 28 hours, covering the cell layer with methylcellulose to restrict viral diffusion. Viral foci are detected with a rabbit anti-spike protein monoclonal antibody, and enumerated automatically. Evaluation then compares the numbers of foci in the absence to the numbers of foci in the presence of plasma (Tx), using valid, reliable and robust statistics. At this point, it becomes obvious that the test primarily measures inhibition of the original encounter of virus with Vero E6 cells, since only the numbers of foci are affected, apparently not their size, as is demonstrated by Fig. 2A. I wonder whether the authors might want to comment on that in the text and whether data are available showing whether indeed the size of foci is affected or not, rather than leaving this to the eyes of the reader. This would be a minor revision.
A second point that needs clarification is whether the patients whose serum has been analysed here had been HIV infected and had received ART treatment or not (page 6, lines 118ff), an antiviral treatment that could have impacted on the response to SARS-CoV-2, both directly and indirectly, by impairing T cell help. The text says that "... most cohort participants had sustained virological suppression ...", does this include the patients analysed here?
A third point is touched by the authors, but rather in passing by (page 6, lines 129ff), namely to what extent neutralisation by serum antibodies indicates protection from infection and/or severe systemic disease? It is obvious that antibodies protecting epithelial cells of the nasypharyngal tract and lung from being infected by SARS-CoV-2 have to be mucosal antibodies, i.e. secretory IgA. So far, to my knowledge, the correlation of secretory IgA in the serum and on mucosal surfaces has not been determined, and this is one of the remaining reservations of the current work. It should at least be discussed. In this context, it might be helpful to show as supplementary data the ELISA data quantifying the serum antibodies binding to the spike protein and its RBD domain in those

patients, data which the authors obviously have, or can generate easily, and at best discriminating between IgM, IgG and IgA, at the very best even secretory IgA (with J-chain). The latter could provide a basis for speculation on the absence of mucosal protection.

Author Rebuttals to Initial Comments:

We thank the referees for the insightful comments. Below is a point-by-point reply to referees' concerns.

Referee #1 (Remarks to the Author):

Although the results are consistent with a series of reports showing that the SA variant is less sensitive to convalescent plasma, the small number of individuals examined, the bias of the cohort to females, and the lack of longitudinal data makes the data difficult to evaluate in the overall context of the pandemic. In conclusion the manuscript feels limited and the data set insufficient to make reliable conclusions.

We have now assayed plasma from 20 participants, 8 of them male. The 95% confidence intervals in PRNT50 for comparisons do not overlap (Figure 3). This indicates that our numbers are sufficient to draw conclusions.

We have used plasma approximately 1-month post-infection, reported to be near the antibody peak response. Durability of the antibody response and how that affects neutralization is a separate question and needs a separate study design.

Additional Problems:

1. The authors "combined the data for both 501Y.V2 variants" before calculating neutralization parameters. As neutralization results for those variants look very different (Fig. S2 B/D), it is not clear why this was done. The authors could have calculated independent ratios to neutralization of the matched 1st wave variant. This would have been especially important since one of the two variants contained a furin cleavage site mutation.

At the time this work was done the clinical trial results have not yet been released and much less was known about the potential of 501Y.V2 to escape. We needed to know if there was a problem, and get the message out if there was. The specific differences between variants were secondary. Our assay at that point was less well calibrated and lacked the more concentrated plasma dilutions, and our fits needed all the data to be accurate.

This is now stated in the legend of Figure S1 which contains the old data: "Data from both 501Y.V2 variants was combined as separate experiments to obtain a more accurate fit of the data using a sigmoidal function since the declines in 501Y.V2 infection were small in the range of plasma concentrations used".

In the revision experiments, we did not use the variant with the in vitro furin site deletion and have the more concentrated plasma dilutions.

2. In addition, it is not clear how the data was combined. The curves in Fig. S2 show overall lower maximum values than those in Figure 2.

See response to point 3.

3. It is unclear whether the 1st wave neutralization profiles were also combined? If so, how did the authors get from Figure S2 A/C to Figure 2B? The yellow curve for example has its maximum at 0.7 in S2, but at 0.9 in Figure 2. The control curve has its maximum at 1.0/0.8 in Fig. S2, but at 1.2 in Figure 2.

We thank the referee for pointing this out, it was an oversight in the supplementary figure description. When we analyzed the dataset from the initial round of experiments, we found that there were lower numbers of foci on the edges of the plate due to edge effects. We identified the edge effect because of the uninfected plasma control, which was on the last row of the plate and gave low FFU. We removed the plates with edge effect from the analysis as part of quality control, and the problem was solved in subsequent experiments by adding sterile water between the wells (now described in the Materials and Methods). We did not perform this quality control in the supplementary figure, which shows all the data. The differences are in the uninfected plasma control values and in the mean 1:100 dilution values for both 501Y.V2 and first wave infections, but the trends are the same.

For the new experimental data, there were no edge effects and every plate done was analyzed and included.

This is now stated in the legend of Figure S1 which contains the previous data:
"The matched infections with first wave virus which were done in parallel with each 501Y.V2 variant were also combined. One experiment was removed in the process of quality control due to plate edge effects, which were subsequently corrected by adding sterile water between wells."

4. There is no doubt that 501Y.V2 neutralization is strongly impaired when looking at the neutralization curves, but given the neutralization curves shown here, it is not clear how accurate the reported titer ratios are (1st wave vs. 501Y.V2 neutralization).

We agree with the referee that in order to obtain added accuracy, the experiments needed to be extended.
We have therefore:

1) Added more concentrated plasma (1:25, 1:50 dilutions).
2) Reduced focus size for 501Y.V2.
3) Added a monoclonal antibody as a positive control to check for assay saturation.

When we designed these experiments in December right after isolating the virus, all we had for detection was a human antibody which gave very dirty readout with the 1:25 and 1:50 plasma dilutions, so we could only obtain the rougher results presented in the original submission. Since then, we were able to optimize our system with rabbit antibody for detection and clarification of the samples to reduce the background (as described in the Materials and Methods), allowing much cleaner readout (see Figure S2). This allowed us to obtain the more accurate results we present here, although the trends remain the same.

5. For the 1st wave virus, the plasma was not diluted out sufficiently to see maximum infection (0% neutralization). This can make the curve-fitting imprecise. For the yellow curve for example, the neutralization range covered is only around 40-85% and this range was then used to define NT50. This effect is even more pronounced when looking at the data in Fig. S2, showing data prior "combining".

This is an important comment, addressed in the previous point. The experiments were repeated and this issue no longer exists.

6. For 501Y.V2 none of the curves even reaches close to 50% neutralization and titers were consequentially extrapolated from curve fits. This extrapolation might make the titer quite

inaccurate which will consequently strongly affect the ratios (neutralization 1st wave vs. neutralization 501Y.V2). The lowest dilution tested is 1:100, the lowest titer reported is 1:3. If this titer in reality is e.g. 1:12, this will reduce the ratio of 1st wave to 501Y.V2 from 200- to 50-fold.

This is an important comment relating to points 4 and 5, and addressed in the response to point 4. The experiments were repeated and this issue no longer exists.

Minor points:

1. Neutralization activity of plasma should be expressed as neutralization titers (NTx) and not as inhibitory concentrations (ICx). The parameter measured is the plasma dilution at which 50
% neutralization was observed, therefore neutralization titers are usually expressed as reciprocal plasma dilutions, e.g. NT50=100 if 50% neutralization was observed at 1:100 plasma dilution.

We have followed this advice and present neutralization as

PRNT50 2. Line 85: 1x10-3 equals 1:1000

This is no longer in the manuscript, but the PRNT50 presented in Figure 2E makes it easier to keep track of the actual dilutions.

3. Figure 2E: colors are swapped

Typo, changed in Figure S1.

Referee #2 (Remarks to the Author):

In their manuscript Cele and colleagues describe the neutralizing capacity of six serum samples against two isolates of B.1.351. Of course, the manuscript is highly significant and the information important, but there are many points that need the authors' attention.

Major points

1) The very limited number of samples and the absence of post-vaccination serum limit the significance of the findings considerably.

We have now assayed plasma from 20 participants. Post-vaccination plasma is addressed by our work elsewhere (Madhi et al., medRxiv 2021.doi:10.1101/2021.02.10.21251247). That work shows a similar 501Y.V2 escape to natural infection presented here.

2) One isolate 'escapes' neutralizing antibody responses much better than the other one. That isolate has also a much bigger plaque phenotype. Better growth characteristics in vitro can easily skew results of neutralization assays but may have no impact in vivo. It would be very very important to make sure that the virus growth kinetics allow simple cross-comparisons. The best would be to use a control that should inhibit all three isolates at a similar level, e.g. remdesivir. That would give much more confidence in the findings. If remdesivir inhibits the HV001 also much less than HVdF002, then it becomes clear that this is caused by an assay artifact.

This is an important point. To rule out saturation, we have

1) Added more concentrated plasma (1:25, 1:50 dilutions) so that a decline in 501Y.V2 infection is now quantifiable.
2) Reduced focus size for 501Y.V2 by reducing infection incubation time to 18 hours.
3) Added a monoclonal antibody to spike which is not affected by 501Y.V2 mutations as a positive control to check for assay saturation.
4) Added plasma from 501Y.V2 infected participants to show the LVNA can read out neutralization for 501Y.V2.

These changes required us to repeat the experiments, and the new experiments are now presented in Figure 2 and 3. A visual check of the raw focus forming assay for each participant plasma is provided in Figure S2.

3) The panels in Figure S2 should be moved to Figure 2 to make sure that these differences are abbreviated.

Figure 2 now contains the redone experiments with more accurate results, but same overall trend. We did not do additional work with the furin cleavage site in vitro deletion mutant.

In lines 175-178 we add the statement:
"The variant we used has an L18F mutation in the NTD which currently occurs in about a quarter of 501Y.V2 variants (GISAID). Other current and future 501Y.V2 variants can be examined to track changes in neutralization and cross-neutralization."

4) Inhibition based on serum dilutions should be reported as ID50, not IC50. The specific antibody concentration in serum is not known (text and figure 2).

We now present the data as PRNT50, the reciprocal of the ID50.

5) The way the inhibition curves are shown is confusing. The dilutions (1:100 etc.) should be shown on the x-axis. % inhibition should be shown on the y-axis.

We have now changed the presentation the neutralization curves and % neutralization is shown on the y-axis. Dilution (log scale) is shown on x-axis. We also present the PRNT50 in Figure 2E to make the absolute values clearer.

6) The trial results from J&J and Novavax in RSA should be discussed.

The AstraZeneca, Novavax and J&J are discussed on lines 46-58, highlighting the loss of VE with 501Y.V2

Minor points

1) Line 48: Define 'ELISA'

Now defined in the Materials and Methods, "Microneutralization using focus forming assay" section.

2) Line 57, 229: 'spike', not

'Spike' All 'Spike' changed to

'spike'.

3) Line 85: Are we sure this calculation is correct? In my book 1x10-3 is 1:1000.

Typo. The PRNT50 graph (Figure 2E) now provides a summary of PRNT50 so that these values are more clear.

4) Figure 2E: With all the variation shown in B and C, how can the error bars here be so low?

There were 6 participants, each with 8 experiments, resulting in a mean of n=48 values per data point. Therefore, STD was divided by √48 to obtain s.e. However, we agree that when data is combined over multiple participants, the standard deviation is more informative, since it shows the variance in neutralization between participant plasma samples. We have therefore used STD when we combined data from participants in Figure 3.

5) Line 125: Please provide a reference for the increase of reinfections.

The re-infection data cited is that reported by the Novavax trial (lines 47-52):
"The Novavax NVX-CoV2373 subunit vaccine demonstrated a decrease in efficacy from 89.3% to 49.4% (https://ir.novavax.com/news-releases/news-release-details/novavax-covid- 19-vaccine-demonstrates-893-efficacy-uk-phase-3). This trial also detected SARS-CoV-2 seroprevalence, and in the placebo arm there was no difference in infection frequency between participants who were seropositive for SARS-CoV-2 relative to those who were negative, indicating that previous infection with first wave, non-501Y.V2 virus does not protect against re-infection with 501Y.V2."

6) Line 126/127: After the second vaccination neutralizing antibody titers are consistently in the top 25% of what is seen with natural infection. Also, based on the titers shown, the convalescent serum used in the study was not very potent.

The referee is correct that the convalescent plasma PRNT50 are in the lower range of what is elicited with BNT162b2. This has now been incorporated in the interpretation of the results on lines 163-165:
"This cross-neutralization is within the lower part of the range elicited by the Pfizer BNT162b2 mRNA vaccine [12, 15, 13]."

7) Many abbreviations, especially in the methods part, are not defined.

Expanded where found.

8) Line 198: 'biosafety', not

'Biosafety' Changed.

9) The way the assay is run the serum concentration used to incubate with the virus before infection of cells is not consistent with the serum concentration present in the overlay during the assay. This is unusual and should be pointed out.

This is now added to the Materials and Methods, lines 289-291:
"For experiments, plasma was serially diluted two-fold from 1:100 to 1:1600, where this is the concentration during the virus-plasma incubation step before addition to cells and during the adsorption step."

Referee #3 (Remarks to the Author):

This is a very important contribution for our understanding of whether the newly arising variants of SARS-CoV-2 escape immunity to the original virus. The data suggest that the

polyclonal plasma antibodies generated in an immune response to the original SARS-CoV-2 virus are significantly less efficient in blocking infection of target cells, when comparing the 501Y variant to the original virus. The finding adds a decisive facet to the recent reports, published as preprints (refs #12 -14 of ms), that monoclonals of convalescent COVID-19 patients, or serum from one such patient, are inefficient in neutralising E484 variants, which would include 501Y. Here, the authors go beyond that and demonstrate that polyclonal convalescent serum of several COVID-19 patients shows little to no efficacy to block infection of VERO E6 cells by the 501Y variant virus. Presumably via the spike protein binding its target ACE2, although this has not been shown directly. Although still epidemiological data are missing, demonstrating that indeed immune responses to the original SARS-CoV-2, or to the existing vaccines, do or do not protect from infection with the 501Y variant, the evidence presented in this manuscript is alarming and should initiate and justify the immediate development of adapted vaccines. In the current situation, the work is of utmost relevance and I highly recommend acceptance, pending a few minor revisions (see below).

We thank the referee for the support and point out that as the referee predicted may happen, indeed vaccine efficacy is compromised with 501Y.V2.

Technically the approach is pretty straightforward. The authors describe outgrowth and isolation of two 501 variants carrying a number of mutations in the RBD and NTD domains of the spike protein, including E484K and N501Y. They then use plasma from blood of convalescent patients, collected at about 1 month after onset of symptoms, diluted at 1:100 to 1:1600, mix it with 50 "focus-forming units" of virus and infect Vero E6 cells for 28 hours, covering the cell layer with methylcellulose to restrict viral diffusion. Viral foci are detected with a rabbit anti-spike protein monoclonal antibody, and enumerated automatically. Evaluation then compares the numbers of foci in the absence to the numbers of foci in the presence of plasma (Tx), using valid, reliable and robust statistics. At this point, it becomes obvious that the test primarily measures inhibition of the original encounter of virus with Vero E6 cells, since only the numbers of foci are affected, apparently not their size, as is demonstrated by Fig. 2A. I wonder whether the authors might want to comment on that in the text and whether data are available showing whether indeed the size of foci is affected or not, rather than leaving this to the eyes of the reader. This would be a minor revision.

The referee is correct that these effects do happen and we have now commented on reduced focus size on lines 108-110:

“Some of the foci were smaller at the higher antibody concentrations (Figure 2C, Figures S2- S4), possibly indicative of some reduction in cell-to-cell spread by neutralizing antibodies in the Vero E6 cell line.”

This may be an important observation, but we will need to follow up on it in a separate study as it shows antibody effects on cell-to-cell spread of the virus, which we think requires validation in a human cell line given that such spread is cell-dependent.

A second point that needs clarification is whether the patients whose serum has been analysed here had been HIV infected and had received ART treatment or not (page 6, lines 118ff), an antiviral treatment that could have impacted on the response to SARS-CoV-2, both directly and indirectly, by impairing T cell help. The text says that "... most cohort participants had sustained virological suppression ...", does this include the patients analysed here?

We now provide HIV status and HIV viral load in Table S1. All included participants were HIV suppressed with an undetectable viral load and the fraction of HIV+ participants, at about a third, is representative of the prevalence in the population.

A third point is touched by the authors, but rather in passing by (page 6, lines 129ff), namely to what extent neutralisation by serum antibodies indicates protection from infection and/or severe systemic disease? It is obvious that antibodies protecting epithelial cells of the nasypharyngal tract and lung from being infected by SARS-CoV-2 have to be mucosal antibodies, i.e. secretory IgA. So far, to my knowledge, the correlation of secretory IgA in the serum and on mucosal surfaces has not been determined, and this is one of the remaining reservations of the current work. It should at least be discussed. In this context, it might be helpful to show as supplementary data the ELISA data quantifying the serum antibodies binding to the spike protein and its RBD domain in those patients, data which the authors obviously have, or can generate easily, and at best discriminating between IgM, IgG and IgA, at the very best even secretory IgA (with J-chain). The latter could provide a basis for speculation on the absence of mucosal protection.

Mucosal protection is an important point but is beyond the scope of this study. We highlight this caveat in lines 179-181:
“Lastly, while we and others in the field measured plasma neutralization, how well this correlates to protection at the mucosal surface where initial infection takes place is yet unclear.”

Regarding neutralization as a correlate of protection, this has not been proven, as the referee observes. Since this work went for review, the clinical trial results came out and in the AstraZeneca trial there was an association between loss of neutralization and loss of vaccine efficacy. T cell immunity may also be involved, but we speculate that it is more difficult for escape mutations to be fixed because of HLA diversity in the population. Thus, an escape variant for one person will not escape T cells in another.

We now discuss this in lines 188-194 of the text:

“The recent Novavax, Johnson and Johnson, and AstraZeneca South African vaccine trial results indicate that the 501Y.V2 variant may lead to a decrease in vaccine efficacy. The loss of neutralization capacity in 501Y.V2 infection we quantified among the vaccinated participants in the AstraZeneca trial [22] shows that loss of neutralization may be associated with loss of vaccine efficacy. Loss of vaccine efficacy may also be mediated by escape from T cell immunity, although we believe this is less likely due to the diversity of HLA alleles in the population, which may curtail the ability of an escape variant which evolved in one individual to escape T cell immunity in another.”

Reviewer Reports on the First Revision:

Referee #1 (Remarks to the Author):

The manuscript is much improved and good to go.

Referee #2 (Remarks to the Author):

This looks very good to me. My only point is that the 'reinfections' in the Novavax trial are a little suspicious. Serological background in African countries to spike/RBD is higher. If Novavax used the same assay setup as in the UK, these may mostly be false positives.

Referee #3 (Remarks to the Author):

This is great! The authors have considerably improved the manuscript and I strongly recommend acceptance. They have appropriately responded to all my criticisms. Manuscript should be accepted now.